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SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

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<211> 654

<212> DNA

<213> Escherichia coli K12

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gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgac 240
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aaaggctaca ccagtctggt ggttggtccg gtaggtcatc acagcgttga agattttaac 600
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<210> 2

<211> 826

<212> DNA

<213> *Pseudomonas putida* JLR11

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agcgatcttc ctgtggatga gcagatgctg agctgggcga tcgcggcggc ccagtcagcc	180
tcgacttcct cgaacctgca agcttgagc gtgctcgccg tgcgggatcg cgagcgtctc	240
gcgaggcttg cccgactgtc cggtaaccag cgccatgtcg agcaggcacc gctgttcctg	300
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gttgctgcct atgaccgaag gatgagcgac ttccaacatc gtcaacaacg cgaaaaccgt	720
tcctgggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cggaagacac	780
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<210> 3

<211> 1066

<212> DNA

<213> *Escherichia coli* K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc_feature

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<222> (160) .. (177)

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<222> (268) .. (285)

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<222> (996) .. (1010)

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Met Gly Ser Ser His His His His His
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162
 His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
 10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Asp
30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258
Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala
45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306
Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu
60 65 70

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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt	354
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val	
75 80 85	
gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt	402
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly	
90 95 100 105	
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg	450
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val	
110 115 120	
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt	498
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val	
125 130 135	
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa	546
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys	
140 145 150	
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa	594
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys	
155 160 165	
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac	642
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn	
170 175 180 185	
gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta	690
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val	
190 195 200	
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg	738
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu	
205 210 215	
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac	786
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His	
220 225 230	
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa	834
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln	
235 240 245	
aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggtat	888
Asn Ile Thr Leu Thr Glu Val	
250 255	
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tgctgttgaa gcttgcgcc gactcgagc accaccacca ccaccactga gatccggctg	1008
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<210> 4

<211> 256

<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

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<222> (250)..(267)

<223> Cys tags

<220>

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<222> (160)..(177)

<223> His tags

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<222> (268)..(285)

<223> primer

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<222> (996)..(1010)

<223> primer

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			20					25					30		

Gly	Ser	Cys	Cys	Cys	Cys	Cys	Cys	Asp	Ile	Ile	Ser	Val	Ala	Leu	Lys
		35					40					45			

Arg	His	Ser	Thr	Lys	Ala	Phe	Asp	Ala	Ser	Lys	Lys	Leu	Thr	Pro	Glu
	50					55					60				

Gln	Ala	Glu	Gln	Ile	Lys	Thr	Leu	Leu	Gln	Tyr	Ser	Pro	Ser	Ser	Thr
65					70					75					80

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Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala
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<210> 5

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<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<222> (88)..(1029)

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<222> (190)..(207)

<223> cys tag

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<222> (936)..(956)

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                1                      5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act      162
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr
10                      15                      20                      25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc      210
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser
                30                      35                      40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca      258
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro
                45                      50                      55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat      306
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

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ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu 125 130 135			498
ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg 140 145 150			546
acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr 155 160 165			594
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agg atg agc gac ttc caa cat cgt caa caa cgc gaa aac cgt tcc tgg Arg Met Ser Asp Phe Gln His Arg Gln Gln Arg Glu Asn Arg Ser Trp 270 275 280			930
tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly 285 290 295			978
aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc			1026

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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg
300 305 310

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atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc 1139
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<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

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<222> (190)..(225)

<223> primer

<220>

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<222> (190)..(207)

<223> cys tag

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<222> (936)..(956)

<223> primer

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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg
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Gly Ser Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys
35 40 45

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Ala Trp Gln Ala Arg Tyr Gly Glu Pro Ala Asn Leu Pro Ala Ala Asp
50 55 60

Thr Val Ile Ala Gln Met Leu Gln His Arg Ser Val Arg Ala Tyr Ser
65 70 75 80

Asp Leu Pro Val Asp Glu Gln Met Leu Ser Trp Ala Ile Ala Ala Ala
85 90 95

Gln Ser Ala Ser Thr Ser Ser Asn Leu Gln Ala Trp Ser Val Leu Ala
100 105 110

Val Arg Asp Arg Glu Arg Leu Ala Arg Leu Ala Arg Leu Ser Gly Asn
115 120 125

Gln Arg His Val Glu Gln Ala Pro Leu Phe Leu Val Trp Leu Val Asp
130 135 140

Trp Ser Arg Leu Arg Arg Leu Ala Arg Thr Leu Gln Ala Pro Thr Ala
145 150 155 160

Gly Ile Asp Tyr Leu Glu Ser Tyr Thr Val Gly Val Val Asp Ala Ala
165 170 175

Leu Ala Ala Gln Asn Ala Ala Leu Ala Phe Glu Ala Gln Gly Leu Gly
180 185 190

Ile Val Tyr Ile Gly Gly Met Arg Asn His Pro Glu Ala Met Ser Glu
195 200 205

Glu Leu Gly Leu Pro Asn Asp Thr Phe Ala Val Phe Gly Met Cys Val
210 215 220

Gly His Pro Asp Pro Ala Gln Pro Ala Glu Ile Lys Pro Arg Leu Ala
225 230 235 240

Gln Ser Val Val Leu His Arg Glu Arg Tyr Glu Ala Thr Glu Ala Glu
245 250 255

Ala Val Ser Val Ala Ala Tyr Asp Arg Arg Met Ser Asp Phe Gln His
260 265 270

Arg Gln Gln Arg Glu Asn Arg Ser Trp Ser Ser Gln Ala Val Glu Arg
275 280 285

Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala
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Leu Asn Thr Leu Gly Phe Gly Leu Arg
 305 310

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<212> DNA

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<210> 8

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<212> DNA

<213> Escherichia coli

<400> 8
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<210> 9

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<212> DNA

<213> Artificial Sequence

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<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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